Final Report

Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice

(Study No. : GT13-00019)

November 2014

the way to trust KCL Korea Conformity Laboratories

Bioconvergence Technology Laboratory

Statement

Study No. : GT13-00019

Title : Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice

This final report was written in Korean and translated into English.

This study has been performed in compliance with the principles of Good Laboratory Practices and test guidelines in following documents.

1. National Institute of Environment Research (NIER) [Notice No. 2012-23, (revised 22 August 2012)].
2. OECD Guidelines for the Testing of Chemicals No. 474 "Mammalian Erythrocyte Micronucleus test"(Adopted : 21st July 1997).

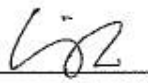
The stated object in study protocol was achieved and there were no significant deviations from the aforementioned regulations that affected quality or integrity of the study. Therefore the justification of all data in this study was confirmed. The information of the test substance was written from the document that study sponsor provided.

14 November 2014

Jin-Sik Kim — Date

Study Director

Bioconvergence Technology Laboratory, Korea Conformity Laboratories



Nov. 14, 2014

Jin-Kyu Lee — Date

Management

Bioconvergence Technology Laboratory, Korea Conformity Laboratories

QUALITY ASSURANCE STATEMENT

Study No. : GT13-00019

Title : Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice

This study was subject to audit by the independent Quality Assurance Unit of KCL as indicated below. The findings of each audit were reported to the study director and management as prescribed by Standard Operating Procedures.

The final report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the final report accurately reflect the raw data produced during the study.

Audit phases and dates reported to the responsible personnel were as indicated below and these were based upon the audit records.

Phase Inspected	Date	Reports to Study Director	Reports to Management
Study Plan	2013. 03. 18	2013. 03. 18	2013. 03. 18
Animal receipt	2013. 05. 14	2013. 05. 14	2013. 05. 14
Storage of test substance and vehicle	2013. 04. 01	2013. 04. 01	2013. 04. 01
Preparation of test substance	2013. 05. 21	2013. 05. 21	2013. 05. 21
Animal care and Administration	2013. 05. 21	2013. 05. 21	2013. 05. 21
Clinical sign	2013. 05. 21	2013. 05. 21	2013. 05. 21
Necropsy and Preparation of specimen	2013. 05. 22	2013. 05. 22	2013. 05. 22
Observation	2013. 05. 22	2013. 05. 22	2013. 05. 22
Raw data	2013. 08. 23	2013. 08. 23	2013. 08. 23
Final report	2013. 08. 23	2013. 08. 23	2013. 08. 23

QA director : Song, Kyung Seuk Ph.D. Date 2013. 08. 23
Auditor, Quality Assurance

* signed original

Study Personnel

Techincal Assistant	Hyo-Jin Joo *	**Date**	23 August 2013
Sample preparation	Jae-Hyuck Sung *	**Date**	23 August 2013
Animal Experiment	Hye-Jin Kim *	**Date**	23 August 2013
Archiving of documents	Hyo-Dong Kim *	**Date**	23 August 2013

* Signed original

Title	Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice.	
Objectives	This test was performed to assess the ability of MWCNT to induce chromosomal aberrations in CHO-k1 cells.	
Sponsor	Name	: Bioconvergence Technology Laboratory, Korea Conformity Laboratories
	Address	: 8, Gaetbeol-ro 145 beongil, Yeonsu-gu, Incheon, Korea
	Tel.	: +82-32-859-4046 Fax : +82-32-858-0020
Testing facility	Name	: Bioconvergence Technology Laboratory, Korea Conformity Laboratories
	Address	: 8, Gaetbeol-ro 145 beongil, Yeonsu-gu, Incheon, Korea
	Tel.	: +82-32-859-4046 Fax : +82-32-858-0020

Study Schedule	Animal aquisition	: 14	May	2013
	Grouping	: 20	May	2013
	Administration	: 21	May	2013
	Slide preparation	: 22	May	2013
	Submission of final report	: 23	August	2013

Archives

1) Period of storage : 5 years after study completion
2) Documents of storage : Study plan, Documents related to the test substance, Raw data, Final report, Documents related to the GLP
3) Room of specimen storage : Slides
4) Room of storage : CD, Related documents

Contents

1. Summary

The test substance, MWCNT, was evaluated for its potential to induce micronucleus in bone marrow of the oral administrated mouse.

Animals were 8 weeks old at administration of test substance. Test animals were dosed once orally with 3 dosing levels (50 ㎎/㎏, 25 ㎎/㎏, 12.5 ㎎/㎏) and then frequency of MNPCEs and cytotoxicity (PCE ratio in the erythrocytes) was determined at 24 hours after administration of test substance. As a results of counting the frequency of MNPCEs of 2,000 PCEs, there was not statistically significant increase at any dose groups compare with negative control group. Also, there was statistically significant increase in positive control group compare with negative control group(p<0.05). The PCEs ratio of 200 erythrocytes (PCE/(PCE+NCE)), as a index of cytotoxicity, was not significantly decrease in PCE/(PCE+NCE) ratio compared to negative control group.

It was concluded that, under the condition of this study, test substance MWCNT did not induce micronucleus in mice bone marrow.

2. Test substance and control substances

1) Test substance

(1) Product name : MWCNT (KUMHO: K-Nanos-100P)

(2) Lot No. : Not available

(3) Received date : 25 January, 2013

(4) Received quantity : 666.89 g (including container weight)

(5) Appearance : Powder

(6) Purity : More than 90 % (We assumed 100 % for test substance and carried out the test)

(7) Solubility : The test substance was dispersed in DPPC solution at 1.0 % concentration.

(8) Storage condition : Room temperature

(9) Stability : Not available

(10) Caution : Not available

(11) Supplier : KUMHO PETROCHEMICAL

2) Negative control substance (solvent 1)

(1) Name : 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

(2) Lot No. : 078K5203

(3) CAS No. : 63-89-8

(4) Molecular weight : 734.04

(5) Date received : 21 March 2012

(6) Quantity Received : 1 g

(7) Appearance : White powder

(8) Grade : ≥ 99 %

(9) Storage condition : Freezing storage

(10) Supplier : Sigma-Aldrich, Inc.

3) Negative control substance (solvent 2)

(1) Name : Dulbecco's phosphate buffered saline (DPBS)

(2) Lot No. : 031M8307

(3) CAS No. : Not available

(4) Date received : 08 May 2012

(5) Quantity Received : 480 g

(6) Appearance : White powder

(7) Storage condition : Cold storage

(8) Supplier : Sigma-Aldrich, Inc.

4) Negative control substance (solvent 3)

(1) Name : D-(+)-Glucose
(2) Lot No. : 071M0145V
(3) CAS No. : 50-99-7
(4) Date received : 28 August 2012
(5) Quantity Received : 1 ㎏
(6) Appearance : White powder
(7) Storage condition : Room temperature
(8) Supplier : Sigma-Aldrich, Inc.

5) Negative control substance (solvent 4)
(1) Name : Bovine serum albumin
(2) Lot No. : 750462
(3) CAS No. : Not available
(4) Date received : 06 April 2009
(5) Quantity Received : 100 g
(6) Appearance : Brown powder
(7) Storage condition : Cold storage
(8) Supplier : Gibco

6) Justification for the selection of solvent

Prior to execution of this study, the solubility of the test substance was not well-dispersed in general dispersion agents. For this reason, we selected the DPPC solution (5.5 mM D-(+)-glucose + 0.6 mg/ml Bovine serum albumin + 0.01 mg/kg DPPC in DPBS) according to Kim et al., 2011 study (Evaluation of biocompatible dispersants for carbon nanotube toxicity tests, Arch. Toxicol. 85: 1499-1508). As a result of solubility test, we observed that the test substance was well-dispersed in DPPC solution at 1.0 % concentration.

7) Positive control substance
(1) Name : Mitomycin C
(2) CAS No. : 50-07-7
(3) Manufacturer : Sigma
(4) Product No. : M-4287
(5) Lot No. : SLBB7481V
(6) Character : Water soluble
(7) Storage condition : 4 ℃
(8) Justification for the selection

Positive control substances were selected according to OECD guidelines No. 474.

8) Storage and treatment (KCL/SAM/013)

During the study, test substances were stored in the storage room No. 108-2. All

storage and treatment conditions are in accordance with the relevant regulations. The stability test and the homogeneity test were not performed because the test solution was prepared fresh in treatment day.

3. Preparation of test substance

The test substance was dispersed in DPPC solution and serially diluted with DPPC solution. The administration level of prepared test substance is determined to 20 ㎖/㎏ based on body weight measured at the administration date. The stability test and the homogeneity test were not performed because the test solution was prepared fresh in treatment day.

4. Test animal

1) Species and strains : SPF (Specific Pathogenic Free), CrljOri:CD1(ICR) mice
2) Supplier : Orient Bio Co., Ltd. (Address ; 143-1, Sangdaewon-dong, Jungwon-gu, Seongnam, Gyeonggi-do, Korea)
3) Reason for animal selection : ICR mice has been widely used in mammalian erythrocyte micronucleus test. It has been known for suitable experimental animal for general toxicity. Moreover, sufficient fundamental data have been accumulated in toxicological study using ICR mice. Such data will be useful for interpretation and evaluation of test results.
4) Date of animal acquisition
Preliminary dose-range finding study : 02 April 2013
Main study : 14 May 2013
5) Number of animal
Preliminary dose-range finding study : 33 male mice
Main study : 33 male mice
6) Age at the arrival : 7 weeks old
7) Quarantine and acclimation
Preliminary dose-range finding study : After 7 days of the acquisition
Main study : After 7 days of the acquisition
(The only healthy animals were selected for this study by observation of general symptoms during the acclimation period)
8) Age of test animal at administration : 8 weeks old
9) Number of test animal
Preliminary dose-range finding study : 30 male mice

Main study : 30 male mice

10) Grouping Method : Animals were weighted one day before the administration, and grouped by using the graded body weight randomly.

11) Identification of individual animals : Individual animals was identified by tail marking using oil marker and individual cages were identified by card labeling. The record sheet was posted at the entrance of animal room and the study number, test title, test period, name of study director was recorded.

12) Weighing method of test animals : Body weight of test animals was measured at the time of animal acquisition, grouping and administration of test substance. Body weight was measured one by one in accordance with individual animal number using electrical balance calibrated by calibration standard weight and recorded in data sheet.

13) Disposal of remaining animals : The remaining animals were sacrificed by mercy killing. They are treated by SOP of this testing facility.

14) Ethics for animal use : This study was approved by the Korea Conformity Laboratories (KCL) Institutional Animal Care and Use Commitee (IA13-00255).

5. Breeding Environment

1) Room Number : SPF Room No. 4 in the SPF animal facility area

2) Range of acceptable temperature and humidity

Preliminary dose-range finding study : 21.8 ± 0.7 ℃ of temperature, 44.9 ± 5.1 % of relative humidity

Main study : 21.5 ± 1.1 ℃ of temperature, 53.8 ± 5.1 % of relative humidity

3) Ventilation frequency : 10～15 times/hr.

4) Lighting cycle : 12 hours of lighting duration (lighting up at 8 a.m.～lighting out at 8 p.m.)

5) Luminous intensity

Preliminary dose-range finding study : 284 Lux

Main study : 284 Lux

6) Breeding cage (size) : During the quarantine, acclimation, administration and observation period, all animals were housed in polycarbonate cage (170 W x 235 L x 125 H ㎜)

7) Animal number per cage

Preliminary dose-range finding study : All animals were housed 5 animals per cage during quarantine and acclimation period and 3 animals per cage during administration and observation period

Main study : All animals were housed 5 animals per cage during quarantine and

acclimation period and 3 animals per cage during administration and observation period

8) Feed, water supply and litter

Feed (Teklad Certified Irradiated Global 18 % Protein Rodent Diet, Harlan Co. Ltd., USA) was supplied from DooYeol Biotech co., Ltd. and were provided free ingestion using feeding supplier. Incheon, Korea municipal tap water purified by reverse osmosis filtering system was provided ad libitum using water polycarbonate bottles. Litter was supplied from DooYeol Biotech co., Ltd. and used after autoclaved.

6. Test method

1) Administration (KCL/AEP/001)

(1) Route of administration

Test substance was administrated by Intraperitoneal (I.P.).

(2) Frequency and duration of administration

Single dose at the morning of administration day

(3) Calculation of dosing volume

Individual dosage is adjusted based on the fasted body weight measured right before the administration. Dosing volume is 20 ㎖/㎏ body weight.

2) Determination of dose level

(1) Preliminary dose-range finding study

Preliminary dose-range finding study was conducted for determination of dose levels and time of harvest in main study. Test groups were consisted of one negative control group, three dosing groups (12.5 mg/kg, 25 mg/kg, 50 mg/kg) and one positive control group (Mitomycin C (MMC) 2.0 mg/kg). Each group was consisted of three mice, harvested at 24 and 48 hours after administration of test substance and conducted for acute toxicity test and cytotoxicity in bone marrow.

(2) Main test

According to determined dose in preliminary study, the highest dose in the main study was 2000 mg/kg. Test groups were consisted of one negative control group (corn oil only), three dosing groups (12.5 mg/kg, 25 mg/kg, 50 mg/kg) and one positive control group (Mitomycin C (MMC) 2.0 mg/kg). Harvest of bone marrow was conducted at 24 hours after administration of test substances. Each group was consisted of six mice.

Group	Sex	Number of animal	Identification of Animal	dosing volume (㎖/kg)
G1 (V.C)	Male	6	1～6	20
G2	Male	6	7～12	20
G3	Male	6	13～18	20
G4	Male	6	19～24	20
G5 (P.C)	Male	6	25～30	10

G1(V.C) : Vehicle Control, G2～G4: Test group, G5(P.C) : Positive Control

3) Reason for determination of dosage

To determined dosage, preliminary study was performed. In the preliminary test, test substance was administrated in dose of 50 mg/kg, 25 mg/kg and 12.5 mg/kg by oral. As the result, There was not observed dead animal and special abnormality compared to negative control group. When observing according to dose level (12.5 mg/kg, 25 mg/kg, 50 mg/kg) and harvest time after administration (24 and 48 hours), no significant decrease in PCE/(PCE+NCE) ratio compared to negative control group was observed (Annex 1). On the basis of preliminary test result, the dose levels in the main study were determined at 50 mg/kg (maximum dose), 25 mg/kg (middle dose) and 12.5 mg/kg (minimum dose) and harvest time was conducted at 24 hours after administration of test substance.

4) Test method

(1) Extraction of bone marrow and preparation of slides

At the appropriate harvest time points, the thigh bone of the test animal was collected with care to avoid blood contamination in autopsy room after putting the cervical vertebra out. The bone marrow was collected in a centrifuge tube by flushing the thigh bone inside with FBS (Hyclone, Lot No. AXC36539, AXC47493) The extracted bone marrow was centrifuged at 1,000 rpm for 5 minutes and then resuspended with small aliquots of FBS after discarding the supernatant. The bone marrow was smeared on a slide glass and then dried at room temperature and fixed in methanol for 5 minutes. Three slides a bone marrow were prepared.

(2) Staining of specimen

For the scoring the PCEs to NCEs ratio, the slides were stained in 4 % Giemsa staining solution. For the scoring the MNPCEs from PCEs, the slides were fixed and then stained with acridine orange (40 ㎍/㎖) and covered with a cover glasses.

(3) Slide analysis

① Observation method

The observation of slides was performed with blind method and PCE to NCE ratio was examined by optical microscope at 400× magnification. The observation of MNPCEs was performed by fluorescence microscope equipped with FITC filter.

② Criteria

The PCE/NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring 200 erythrocytes per animal. The micronuclei frequency (expressed as percent micronucleated cells) was determined by analyzing the number of MNPCEs from 2,000 PCEs per animal. In case of observation by fluorescence microscope, PCE was counted cells which appeared as red-fluorescence color without nucleus by acridine orange staining. NCE stained with acridine orange was appeared as only shadow entity without fluorescence. In case of observation by optical microscope, PCE stained with Giemsa staining solution was counted cells appeared as purple or blue color. NCE stained with Giemsa staining solution was counted cells appeared as pink color. According to criteia in micronucleus judgement, the largest size was defined as 1/2 size of erythrocyte diameter and the smallest size was defined as up to the limit of identification. Shape was Including circle, donut, semi-circle. Color was defined as same color as nucleus of cell.

(4) Observation of general symptoms

It was conducted once at the administration date and autopsy date for observation of dead and abnormal sign of test animals

(5) Measurement of body weight

It was measured at the administration date and autopsy date.

5) Statistical analysis

(1) Micronuclei frequency and PCE/(PCE+NCE) ratio

① Comparison of the negative control and treated groups: ANOVA test

② In case of $p<0.05$ at 1), test for dose-response : Linear logistic regression

③ The negative and positive control groups : ANOVA test

(2) Body weight

① Body weight of test animal at necropsy : ANOVA test

② In case of significant difference at ① : Dunnett T3 or Duncan's multiple range test

6) Criteria

In case PCE/(PCE+NCE) ratio (Mean±SD) is more than 0.2 at all the animals, test is judged to be reasonable. Determined as a positive result if there is dose-related increase in the number of micronucleated cells or clear increase in the number of

micronucleated cells in the single dose group and statistical significant increase in frequency of MNPCE(MNPCE/2000PCEs, Mean±SD, %). The result of statistical evaluation was regarded as significant when the P value was less than 0.05. Statistical analysis was performed with SPSS 12.0 program.

7. Results

1) Micronuclei frequency and cytotoxicity (Table 1. and Appendix 1.)
The frequencies of MNPCEs in 2,000 PCEs per animal were 0.08 %, 0.09 %, 0.08 %, 0.04 % and 5.73 % in order of negative control group, 12.5, 25, 50 mg/kg administration group and positive control group. There was no statistically increase in frequency of PCEs having micronuclei in administration group compared to negative control group. There was clear statistically significant increase in positive control group compared with negative control group in frequency of MNPCE (p<0.05).
The PCE ratio of 200 erythrocytes (PCE+NCE), as a index of cytotoxicity, was 0.39, 0.36, 0.36, 0.36, 0.41 and 0.28 in order of negative control, 12.5, 25, 50 mg/kg group and positive control. There was no significant decrease in PCE/(PCE+NCE) ratio compared to negative control group.

2) Clinical sign
As a result of observation, no special abnormality was observed compared to negative control group at necropsy date.

3) Body weight (Table 2. and Appendix 2.)
There was no statistically change in body weight at any group compared to negative control group.

8. Discussion and conclusion

The mammalian erythrocyte micronucleus test was performed to evaluate genotoxicity of the test substance MWCNT using male mouse (ICR mouse).

Animals (ICR mouse) were 7 weeks old at acquisition and acclimated for more than 5 days before treatment of test substance. Test substance was dispersed in distilled water. The dose levels administered to the animals were as follows;

Preliminary does-range finding study : 500, 1000, 2000 mg/kg
Main study : 12.5, 25, 50 mg/kg

In preliminary dose-range finding study, there was not observed death of test animal in all dose level (12.5, 25, 50 mg/kg**)**. When observing according to the dose level and for the harvest times (24, 48 hours) after administration, PCE/(PCE+NCE) ratio was not observed significant decrease in all administrated groups (12.5, 25, 50 mg/kg**)** compared

with negative control group.
In main study, the highest dose was 2000 mg/kg and harvest time point was 24 hours respectively which was referred to result of preliminary dose-range finding study.
There was no statistically significant in frequency of 2,000 PCEs having micronuclei in all test substance groups compared to negative control group. On the other hand, positive control group appeared statistically significant increase compared with negative control group (p<0.05). Also, there was no significant decrease in the number of PCEs per PCE+NCE was observed at the all dose levels (12.5, 25, 50 mg/kg) administered to the animals as compared to negative control.

Statistically change in body weight was not observed in all test group compared to negative control group.

It was concluded that, under the condition of this study, test substance MWCNT did not induce micronucleus in mice bone marrow.

9. References

1) National Institute of Environment Research (NIER) [Notice No. 2012-23, (revised 22 August 2013)].
2) OECD Guidelines for the Testing of Chemicals No. 474 "Mammalian Erythrocyte Micronucleus test"(Adopted : 21st July 1997)
3) Schmid, W. (1975). The Micronucleus Test. Mutation Res. 31: 9-15.
4) 林 眞 (1991). 小核試驗. -實驗法から データの 評價まで 一 醫藥安全性研究會 monograph series No. 2. サイエンテイスト社

10. Tables (Group Summary)

Table 1. Test results (main study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	MNPCE/2000 PCEs (Mean±SD, %)		PCE/(PCE+NCE) (Mean±SD)	
24	Vehicle control	0	1	0.1		0.34	
			2	0.1		0.40	
			3	0		0.41	
			4	0.25		0.44	
			5	0		0.37	
			6	0	(0.08 ± 0.10)	0.36	(0.39 ± 0.04)
	MWCNT	12.5	7	0.15		0.34	
			8	0.05		0.33	
			9	0.1		0.35	
			10	0.1		0.37	
			11	0.05		0.41	
			12	0.1	(0.09 ± 0.04)	0.36	(0.36± 0.03)
		25	13	0.05		0.32	
			14	0.1		0.41	
			15	0.15		0.40	
			16	0		0.33	
			17	0.05		0.34	
			18	0.1	(0.08 ± 0.05)	0.37	(0.36 ± 0.04)
		50	19	0		0.51	
			20	0.05		0.38	
			21	0		0.50	
			22	0		0.44	
			23	0.1		0.37	
			24	0.1	(0.04 ± 0.05)	0.27	(0.41± 0.09)
	MMC	2.0	25	5.15		0.33	
			26	5.25		0.29	
			27	4.9		0.28	
			28	7.2		0.26	
			29	7.85		0.24	
			30	4	(5.73 ± 1.48)*	0.27	(0.28 ± 0.03)*

Vehicle : DPPC solution
* Significantly different from the control at $P<0.05$ (One-way ANOVA)
Abbreviations
MNPCE : PCE with one or more micronuclei
PCE : Polychromatic erythrocyte
NCE : Normochromatic erythrocyte
MMC : Mitomycin C

Table 2. Body weights of animals (main study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	Body weights (gram, Mean±S.D)	
				Administration	Sacrifice
24	Vehicle control	0	6	33.67±1.22	33.80±1.37
	MWCNT	12.5	6	33.51±1.08	32.64±1.05
		25	6	33.55±0.90	32.43±0.79
		50	6	33.22±0.60	33.10±0.50
	MMC	2.0	6	33.08±0.76	33.26±0.66

Vehicle : DPPC solution
MMC : Mitomycin C

11. Appendices (Individual data)

Appendix 1. Test results (main study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	Slide No.	MNPCEs	PCEs	No. of PCE/NCE		Ratio
24	Vehicle control	0	1	19	2	2000	67	133	0.34
			2	30	2	2000	80	120	0.40
			3	5	0	2000	82	118	0.41
			4	23	5	2000	88	112	0.44
			5	3	0	2000	74	126	0.37
			6	29	0	2000	72	128	0.36
	MWCNT	12.5	7	14	3	2000	68	132	0.34
			8	4	1	2000	66	134	0.33
			9	22	2	2000	70	130	0.35
			10	6	2	2000	74	126	0.37
			11	20	1	2000	82	118	0.41
			12	15	2	2000	71	129	0.36
		25	13	10	1	2000	64	136	0.32
			14	25	2	2000	81	119	0.41
			15	21	3	2000	80	120	0.40
			16	1	0	2000	65	135	0.33
			17	18	1	2000	67	133	0.34
			18	13	2	2000	73	127	0.37
		50	19	26	0	2000	101	99	0.51
			20	2	1	2000	76	124	0.38
			21	12	0	2000	100	100	0.50
			22	17	0	2000	87	123	0.44
			23	7	2	2000	74	126	0.37
			24	28	2	2000	54	146	0.27
	MMC	2.0	25	11	103	2000	65	135	0.33
			26	16	105	2000	58	142	0.29
			27	24	98	2000	56	144	0.28
			28	8	144	2000	52	148	0.26
			29	27	157	2000	47	153	0.24
			30	9	80	2000	54	146	0.27

Vehicle : DPPC solution

Abbreviations

MNPCE : PCE with one or more micronuclei

PCE : Polychromatic erythrocyte

NCE : Normochromatic erythrocyte

MMC : Mitomycin C

Appendix 2. Body weights of animals (main study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	Body weight (gram)			
				Administration		Sacrifice	
24	Vehicle control	0	1	31.65		31.38	
			2	34.15		34.61	
			3	33.18		33.37	
			4	33.48		33.71	
			5	34.39		34.35	
			6	35.19	33.67±1.22	35.35	33.80±1.37
	MWCNT	12.5	7	32.81		32.14	
			8	32.33		31.84	
			9	32.62		31.39	
			10	34.08		33.25	
			11	34.20		32.96	
			12	35.03	33.51±1.08	34.25	32.64±1.05
		25	13	31.95		31.43	
			14	33.51		32.25	
			15	33.50		32.08	
			16	33.91		33.71	
			17	33.68		32.18	
			18	34.72	33.55±0.90	32.91	32.43±0.79
		50	19	32.64		32.45	
			20	32.36		32.50	
			21	33.35		33.17	
			22	33.36		33.42	
			23	33.89		33.56	
			24	33.72	33.22±0.60	33.48	33.10±0.50
	MMC	2	25	32.47		32.79	
			26	32.42		32.59	
			27	33.06		32.97	
			28	33.35		33.60	
			29	32.75		33.19	
			30	34.45	33.08±0.76	34.41	33.26±0.66

Vehicle : DPPC solution
MMC : Mitomycin C

12. Annexes

Annex 1. Test results (preliminary range-finding study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	MNPCE/2000 PCEs (Mean±SD,%)		PCE/(PCE+NCE) (Mean±SD)	
24	Vehicle control	0	1	0.1		0.45	
			2	0.15		0.41	
			3	0.25	0.17±0.08	0.39	0.42±0.03
	MWCNT	12.5	4	0.1		0.46	
			5	0.1		0.45	
			6	0.15	0.12±0.03	0.42	0.44±0.02
		25	7	0.05		0.31	
			8	0.05		0.44	
			9	0.15	0.08±0.06	0.49	0.41±0.10
		50	10	0.1		0.45	
			11	0		0.45	
			12	0.1	0.07±0.06	0.32	0.40±0.08
	MMC	2	13	5.25		0.42	
			14	5.6		0.45	
			15	5.55	5.47±0.19*	0.50	0.45±0.04
48	Vehicle control	0	16	0.1		0.41	
			17	0.25		0.47	
			18	0.25	0.20±0.09	0.42	0.43±0.03
	MWCNT	12.5	19	0.2		0.48	
			20	0.1		0.51	
			21	0.05	0.12±0.08	0.47	0.49±0.02
		25	22	0		0.54	
			23	0.25		0.48	
			24	0.1	0.12±0.13	0.38	0.47±0.08
		50	25	0.05		0.46	
			26	0.1		0.38	
			27	0.05	0.07±0.03	0.47	0.43±0.05
	MMC	2	28	3.65		0.46	
			29	3.6		0.25	
			30	5.6	4.28±1.14*	0.26	0.32±0.12

Vehicle : DPPC solution

* Significantly different from the control at $P<0.05$ (One-way ANOVA)

Abbreviations

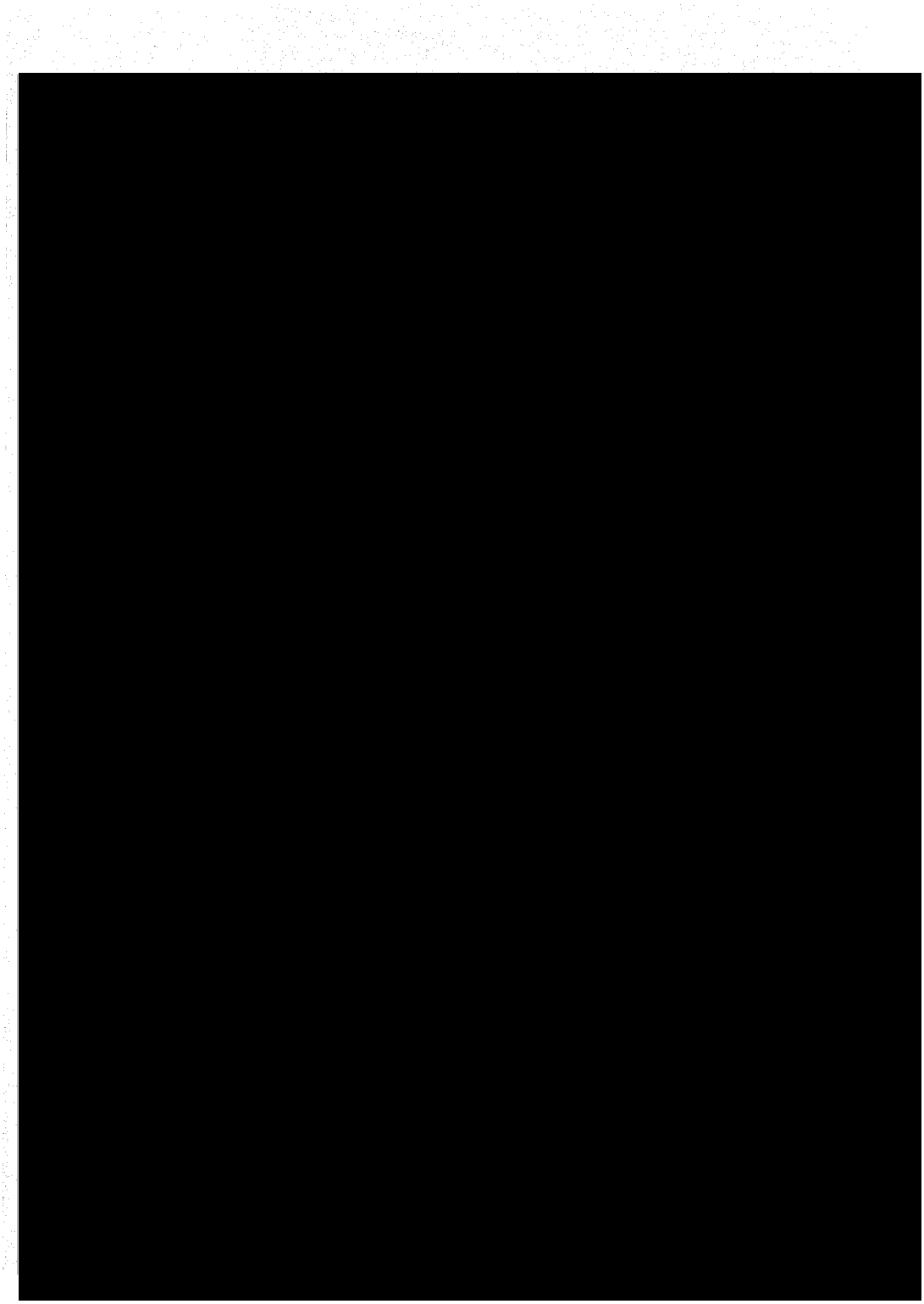
MNPCE : PCE with one or more micronuclei
PCE : Polychromatic erythrocyte
NCE : Normochromatic erythrocyte
MMC : Mitomycin C

Annex 2. Body weights of animals (preliminary range-finding study)

Sampling time(hrs)	Groups	Dose (mg/kg)	Animal No.	Body weight (gram) Administration		Sacrifice	
24	Vehicle control	0	1	31.82		32.28	
			2	33.26		33.01	
			3	34.06	33.05±1.14	35.00	33.43±1.41
	MWCNT	12.5	4	31.05		31.08	
			5	33.41		33.09	
			6	33.26	32.57±1.32	32.87	32.35±1.10
		25	7	32.92		33.17	
			8	32.95		31.65	
			9	33.78	33.22±0.49	34.11	32.98±1.24
		50	10	32.36		30.60	
			11	32.54		30.87	
			12	34.72	33.21±1.31	33.08	31.52±1.36
	MMC	2	13	32.24		32.27	
			14	32.71		33.05	
			15	33.74	32.90±0.77	34.14	33.15±0.94
48	Vehicle control	0	16	32.53		32.80	
			17	32.68		33.29	
			18	34.65	33.29±1.18	33.81	33.30±0.51
	MWCNT	12.5	19	31.73		31.79	
			20	32.43		32.78	
			21	34.11	32.76±1.22	33.95	32.84±1.08
		25	22	32.60		32.44	
			23	32.00		32.38	
			24	33.93	32.84±0.99	33.65	32.82±0.72
		50	25	32.15		31.17	
			26	32.60		32.09	
			27	33.70	32.82±0.80	33.33	32.20±1.08
	MMC	2	28	31.96		32.69	
			29	32.22		32.44	
			30	35.09	33.09±1.74	35.74	33.62±1.84

Vehicle : DPPC solution
MMC : Mitomycin C

Annex 3. Test substance data sheet



Annex 4. Certificate of drinking water analysis

TEST REPORT

1. No : PC12-00576
2. Client

Reissuance (R1)
Date : 2012.10.30

- Name : Korea Conformity Laboratories(Incheon)
- Address : #7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea
- Date of Receipt : Sep. 25, 2012
- Date of Issued : Oct. 30, 2012

3. Use of Report : Submission
4. Test Sample : Drinking Water (Animal room)
5. Test Results

----- Refer to the next page -----

Affirmation	Tested by Name : Hyoung jun Seok	Technical Manager Name : Sang Cheul Lee

Our report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified.
The test results are not indicative of representative of the qualities of the lot from which the sample was taken or of apparently identical or similar products.

Korea Conformity Laboratories President Jae Bin Song

Address : 153-803 459-28, Gasan-Dong, Geumcheon-Gu, Seoul, Korea 82-2-2102-2500
Result Inquiry : 82-2-2102-2598

QP-20-01-07(1)

No : PC12-00576

TEST REPORT

Test Items	Units	Limitations	LOQ	Test Results
Total colony counts	CFU/mL	Less than 100	0	34
Total coliforms	-/(100mL)	Not detected	-	Not detected
E-Coli	-/(100mL)	Not detected	-	Not detected
Lead	mg/L	Less than 0.01	0.005	Not detected
Arsenic	mg/L	Less than 0.01	0.005	Not detected
Selenium	mg/L	Less than 0.01	0.005	Not detected
Cadmium	mg/L	Less than 0.005	0.002	Not detected
Bolon	mg/L	Less than 1.0	0.01	Not detected
Copper	mg/L	Less than 1.0	0.008	Not detected
Zinc	mg/L	Less than 3.0	0.002	0.003
Iron	mg/L	Less than 0.3	0.05	Not detected
Manganese	mg/L	Less than 0.3	0.005	Not detected
Aluminium	mg/L	Less than 0.2	0.02	Not detected
Mercury	mg/L	Less than 0.001	0.001	Not detected
Fluoride	mg/L	Less than 1.5	0.15	Not detected
Nitrate nitrogen	mg/L	Less than 10	0.1	0.2
Chloride	mg/L	Less than 250	0.4	Not detected
Sulfate	mg/L	Less than 200	2	Not detected
Diazinon	mg/L	Less than 0.02	0.000 5	Not detected
Parathion	mg/L	Less than 0.06	0.000 5	Not detected
Fenitrotion	mg/L	Less than 0.04	0.000 5	Not detected
Dichloromethane	mg/L	Less than 0.02	0.002	Not detected
1,1,1-Trichloroethane	mg/L	Less than 0.1	0.001	Not detected
Benzene	mg/L	Less than 0.01	0.001	Not detected
Toluene	mg/L	Less than 0.7	0.001	Not detected
Ethylbenzene	mg/L	Less than 0.3	0.001	Not detected
Xylene	mg/L	Less than 0.5	0.001	Not detected
1,1-Dichloroethylene	mg/L	Less than 0.03	0.001	Not detected
Tetrachlorocarbon	mg/L	Less than 0.002	0.001	Not detected
Tetrachloroethylene	mg/L	Less than 0.01	0.001	Not detected
Trichloroethylene	mg/L	Less than 0.03	0.001	Not detected
1,2-Dibromo-3-Chloropropane	mg/L	Less than 0.003	0.001	Not detected

No : PC12-00576

TEST REPORT

Test Items	Units	Limitations	LOQ	Test Results
Carbaryl	mg/L	Less than 0.07	0.005	Not detected
Chromium	mg/L	Less than 0.05	0.003	Not detected
Ammonia nitrogen	mg/L	Less than 0.5	0.01	Not detected
Phenol	mg/L	Less than 0.005	0.005	Not detected
Detergent	mg/L	Less than 0.5	0.1	Not detected
Cyanide	mg/L	Less than 0.01	0.01	Not detected
pH	-	5.8 ~ 8.5	-	6.4
Turbidity	NTU	Less than 1	0.02	0.16
Color	degree	Less than 5	1	Not detected
Taste	-	Free	-	Pass
Odor	-	Free	-	Pass
Hardness	mg/L	Less than 300	1	Not detected
Consumption of $KMnO_4$	mg/L	Less than 10	0.3	0.9
Total solids	mg/L	Less than 500	2	4
Test method	Notification No.2012-143 of the Ministry of Environment			

—— End of Report ——

TEST REPORT

Reissuance (R1)
Date : 2013.4.11

1. No : PC13-00284
2. Client
   - Name : Korea Conformity Laboratories(Incheon)
   - Address : #7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea
   - Date of Receipt : Mar. 14, 2013
   - Date of Issued : Apr. 17, 2013
3. Use of Report : Submission
4. Test Sample : Drinking Water (Animal room)
5. Method :
   (1) Notification No.2012-143 of the Ministry of Environment

Affirmation	Tested By Name : Hyoung jun Seok Seok.	Technical Manager Name : Sang Cheul Lee S. C. Lee

The report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified.
The test results are not indicative of representative of the qualities of the qualities of the lot from which the sample was taken or of apparently identical or similar products.

Korea Conformity Laboratories President Song Jae Bin Jae Bin Song

Address : 704-932 277-5, Jukjeon-Dong, Dalseo-Gu, Daegu,704-932,Korea 82-53-557-6681
Result Inquiry : Environmental Testing Center 82-2-2102-2598

TEST REPORT

No : PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Total colony counts	CFU/mL	Less than 100	0	(1)	0
Total coliforms	-/(100mL)	Not detected	-	(1)	Not detected
E-Coli	-/(100mL)	Not detected	-	(1)	Not detected
Lead	mg/L	Less than 0.01	0.005	(1)	Not detected
Arsenic	mg/L	Less than 0.01	0.005	(1)	Not detected
Selenium	mg/L	Less than 0.01	0.005	(1)	Not detected
Cadmium	mg/L	Less than 0.005	0.002	(1)	Not detected
Bolon	mg/L	Less than 1.0	0.01	(1)	Not detected
Copper	mg/L	Less than 1.0	0.008	(1)	Not detected
Zinc	mg/L	Less than 3.0	0.002	(1)	0.003
Iron	mg/L	Less than 0.3	0.05	(1)	Not detected
Manganese	mg/L	Less than 0.3	0.005	(1)	Not detected
Aluminium	mg/L	Less than 0.2	0.02	(1)	Not detected
Mercury	mg/L	Less than 0.001	0.001	(1)	Not detected
Fluoride	mg/L	Less than 1.5	0.15	(1)	Not detected
Nitrate nitrogen	mg/L	Less than 10	0.1	(1)	0.2
Chloride	mg/L	Less than 250	0.4	(1)	0.6
Sulfate	mg/L	Less than 200	2	(1)	Not detected
Diazinon	mg/L	Less than 0.02	0.0005	(1)	Not detected
Parathion	mg/L	Less than 0.06	0.0005	(1)	Not detected
Fenitrotion	mg/L	Less than 0.04	0.0005	(1)	Not detected
Dichloromethane	mg/L	Less than 0.02	0.002	(1)	Not detected
1,1,1-Trichloroethane	mg/L	Less than 0.1	0.001	(1)	Not detected
Benzene	mg/L	Less than 0.01	0.001	(1)	Not detected
Toluene	mg/L	Less than 0.7	0.001	(1)	Not detected
Ethylbenzene	mg/L	Less than 0.3	0.001	(1)	Not detected
Xylene	mg/L	Less than 0.5	0.001	(1)	Not detected
1,1-Dichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
Tetrachlorocarbon	mg/L	Less than 0.002	0.001	(1)	Not detected
Tetrachloroethylene	mg/L	Less than 0.01	0.001	(1)	Not detected

TEST REPORT

No : PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Trichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
1,2-Dibromo-3-Chloropropane	mg/L	Less than 0.003	0.001	(1)	Not detected
Carbaryl	mg/L	Less than 0.07	0.005	(1)	Not detected
Chromium	mg/L	Less than 0.05	0.03	(1)	Not detected
Ammonium Nitrogen	mg/L	Less than 0.5	0.01	(1)	Not detected
Phenol	mg/L	Less than 0.005	0.005	(1)	Not detected
Alkyl Benzene Sulfate	mg/L	Less than 0.5	0.1	(1)	Not detected
Cyanide	mg/L	Less than 0.01	0.01	(1)	Not detected
pH	-	5.8 - 8.5	-	(1)	6.2
Turbidity	NTU	Less than 1	0.02	(1)	0.11
Color	degree	Less than 5	1	(1)	Not detected
Taste	-	Free	-	(1)	Pass
Odor	-	Free	-	(1)	Pass
Hardness	mg/L	Less than 300	1	(1)	Not detected
Consumption of $KMnO_4$	mg/L	Less than 10	0.3	(1)	0.6
Total solids	mg/L	Less than 500	2	(1)	Not detected

—— End of Report ——

Page 3 of 3

QP-2(1)-01-08(2)

Annex 5. Laboratory diet certification report

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-120212MA
Date of Manufacture 12/02/12
Report Date 12/18/12

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2012.12.21 07:18:28 -06'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.40
Fat	6.14
Fiber	3.32
Moisture	12.00
Ash	5.51
Calcium	0.98
Phosphorus	0.68

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.17	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.24	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-030413MA
Date of Manufacture 03/04/13
Report Date 03/19/13

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Kurt Schafer
Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc

I have reviewed this document
2013.03.20 09:52:32 -05'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.20
Fat	6.17
Fiber	3.82
Moisture	10.50
Ash	5.66
Calcium	1.01
Phosphorus	0.77

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.12	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.34	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Annex 6. Diagnostic summary report of test animals

Mouse VAF Report

CR Rodent Production

Location: Orient Bio Inc. KP100 VAF Mice

Sponsor: Orient Bio Inc.

Colony: CrljOri:CD1(ICR) Colony # 1358

Reported: Monday, March 4, 2013 at 1:17

Summary Item	Primary Assay	Most Recent		Past 18 Months
		Year-Week	Positive / Tested	Positive / Tested
Virology				
SEND ae	MFIA	2013-05	0 / 8	0 / 48
PVM ae	MFIA	2013-05	0 / 8	0 / 48
MHV ae	MFIA	2013-05	0 / 8	0 / 48
MVM ae	MFIA	2013-05	0 / 8	0 / 48
MPV ae	MFIA	2013-05	0 / 8	0 / 48
MNV ae	MFIA	2013-05	0 / 8	0 / 48
TMEV (GDVII) ae	MFIA	2013-05	0 / 8	0 / 48
REO ae	MFIA	2013-05	0 / 8	0 / 48
EDIM ae	MFIA	2013-05	0 / 8	0 / 48
LCMV ae	MFIA	2013-05	0 / 8	0 / 48
ECTRO ae	MFIA	2013-05	0 / 8	0 / 48
MAV ae	MFIA	2013-05	0 / 8	0 / 48
MCMV ae	MFIA	2013-05	0 / 8	0 / 48
K ae	MFIA	2013-05	0 / 8	0 / 48
POLY ae	MFIA	2013-05	0 / 8	0 / 48
HANT ae	MFIA	2013-05	0 / 8	0 / 48
MTLV ae	MFIA	2013-05	0 / 8	0 / 48
LDV af	MFIA	2012-41	0 / 8	0 / 16
Microbiology				
B. bronchiseptica bc	Culture	2013-05	0 / 8	0 / 48
CAR Bacillus ae	MFIA/PCR	2013-05	0 / 8	0 / 48
C. rodentium ae	Culture	2013-05	0 / 8	0 / 36
C. kutscheri ae	Culture	2013-05	0 / 8	0 / 48
H. bilis bc	PCR	2013-05	0 / 8	0 / 48
H. hepaticus ae	PCR	2013-05	0 / 8	0 / 48
Helicobacter sp. bc	PCR	2013-05	0 / 8	0 / 48
K. oxytoca ae	Culture	2013-05	0 / 8	0 / 48
K. pneumoniae ae	Culture	2013-05	0 / 8	0 / 48
M. pulmonis ad	MFIA	2013-05	0 / 8	0 / 48
P. multocida ae	Culture	2013-05	0 / 8	0 / 48
P. pneumotropica ae	Culture	2013-05	0 / 8	0 / 48
P. aeruginosa ae	Culture	2013-05	0 / 8	0 / 48
Salmonella spp. ae	Culture	2013-05	0 / 8	0 / 48
Staph. aureus ae	Culture	2013-05	0 / 8	0 / 48
S. moniliformis ai	PCR	2013-05	0 / 8	0 / 32
Strep. pneumoniae bc	Culture	2013-05	0 / 8	0 / 48
Tyzzer's Disease ag	Exam	2013-05	0 / 8	0 / 48
Pathology				
Gross Exam eh	Exam, Histopathology	2013-05	0 / 8	0 / 48
Parasitology				
Ectoparasites ae	Exam	2013-05	0 / 8	0 / 48
Helminths ae	Exam	2013-05	0 / 8	0 / 48
Giardia sp. bc	Exam	2013-05	0 / 8	0 / 28
Spironucleus sp. bc	Exam	2013-05	0 / 8	0 / 28
Other Protozoa ae	Exam	2013-05	0 / 8	0 / 28
E. cuniculi ae	MFIA	2013-05	0 / 8	0 / 48

COLONY POLICY FOR POSITIVE RESULT: a = immediate termination; b = planned future recycle of the colony; c = no action.
TESTING SCHEDULE: d = screened every four weeks; e = screened quarterly; f = screened annually; g = screened quarterly by clinical exam.
h = results do not include incidental or strain related findings; significant findings would result in immediate termination of the colony.

Charles River RADS-ILIMS Report

Page 1 of 1

Annex 7. Certificate of animal room

Certification of Environment for animal breeding room

Study No.	GT13-00019
Title	Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice (Preliminary test)
SPF Room No.	SPF #4 Animal Room
Period of animal Breeding	2013-04-02 ~ 2014-04-11

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	21.8±0.7 ℃	
Humidity	50±20 %RH	44.9±5.1% RH	
Luminous intensity	150~300 Lux	284 Lux	
Noise	60 db less than	49.2 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-07-31

Certification of Environment for animal breeding room

Study No.	GT13-00019
Title	Mammalian Erythrocyte Micronucleus Test of MWCNT in ICR mice (Main test)
SPF Room No.	SPF #4 Animal Room
Period of animal Breeding	2014-05-14 ~ 2014-05-22

Breeding environment condition

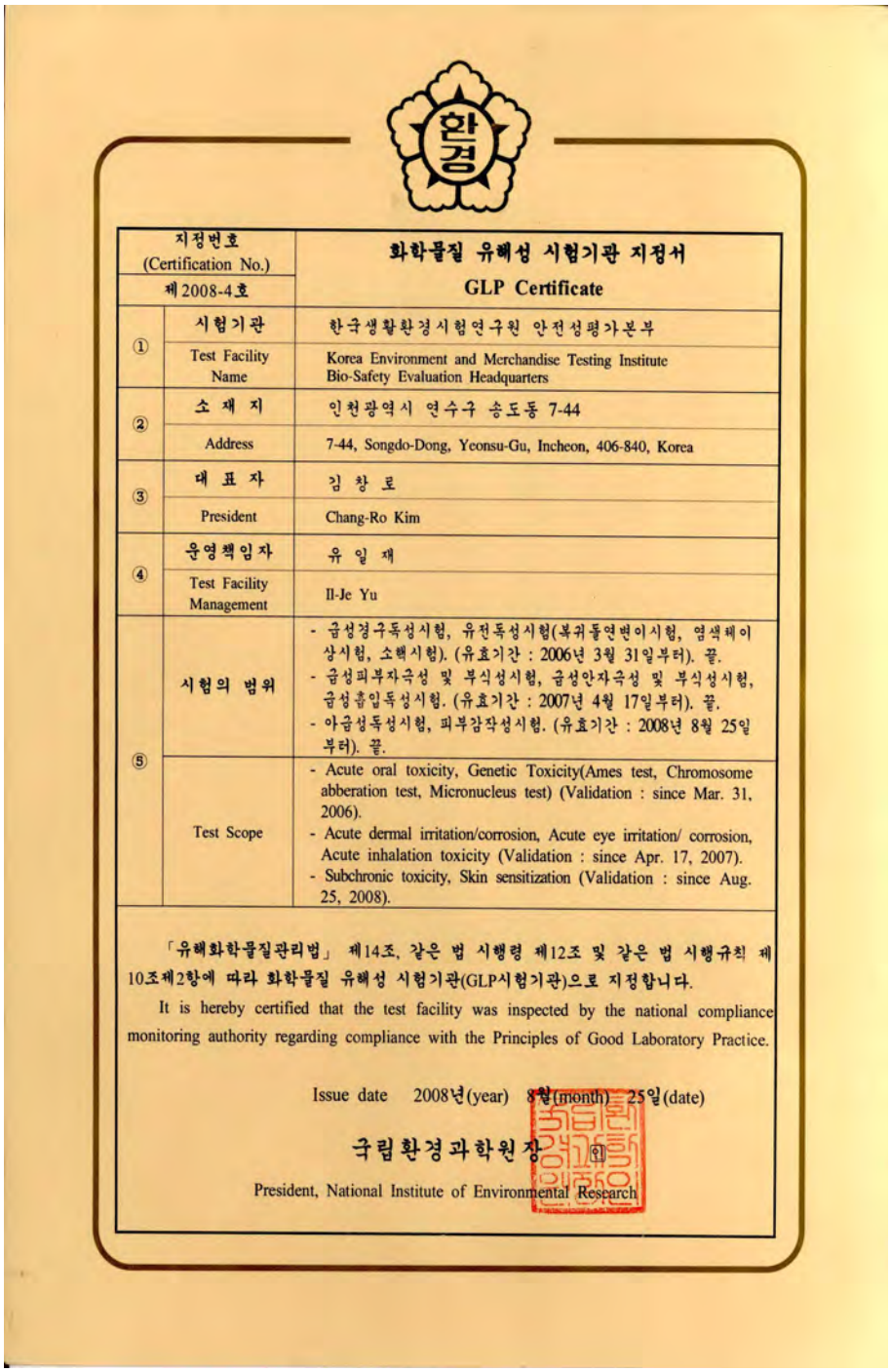
Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	21.5±1.1 ℃	
Humidity	50±20 %RH	53.8±5.1% RH	
Luminous intensity	150~300 Lux	284 Lux	
Noise	60 db less than	49.2 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-07-31

Annex 8. GLP Certificate



지정번호 (Certification No.) 제2008-4호		화학물질 유해성 시험기관 지정서 GLP Certificate
①	시험기관	한국생활환경시험연구원 안전성평가본부
	Test Facility Name	Korea Environment and Merchandise Testing Institute Bio-Safety Evaluation Headquarters
②	소 재 지	인천광역시 연수구 송도동 7-44
	Address	7-44, Songdo-Dong, Yeonsu-Gu, Incheon, 406-840, Korea
③	대 표 자	김 창 로
	President	Chang-Ro Kim
④	운영책임자	유 일 재
	Test Facility Management	Il-Je Yu
⑤	시험의 범위	- 급성경구독성시험, 유전독성시험(복귀돌연변이시험, 염색체이상시험, 소핵시험). (유효기간 : 2006년 3월 31일부터). 끝. - 급성피부자극성 및 부식성시험, 급성안자극성 및 부식성시험, 급성흡입독성시험. (유효기간 : 2007년 4월 17일부터). 끝. - 아급성독성시험, 피부감작성시험. (유효기간 : 2008년 8월 25일부터). 끝.
	Test Scope	- Acute oral toxicity, Genetic Toxicity(Ames test, Chromosome abberation test, Micronucleus test) (Validation : since Mar. 31, 2006). - Acute dermal irritation/corrosion, Acute eye irritation/ corrosion, Acute inhalation toxicity (Validation : since Apr. 17, 2007). - Subchronic toxicity, Skin sensitization (Validation : since Aug. 25, 2008).

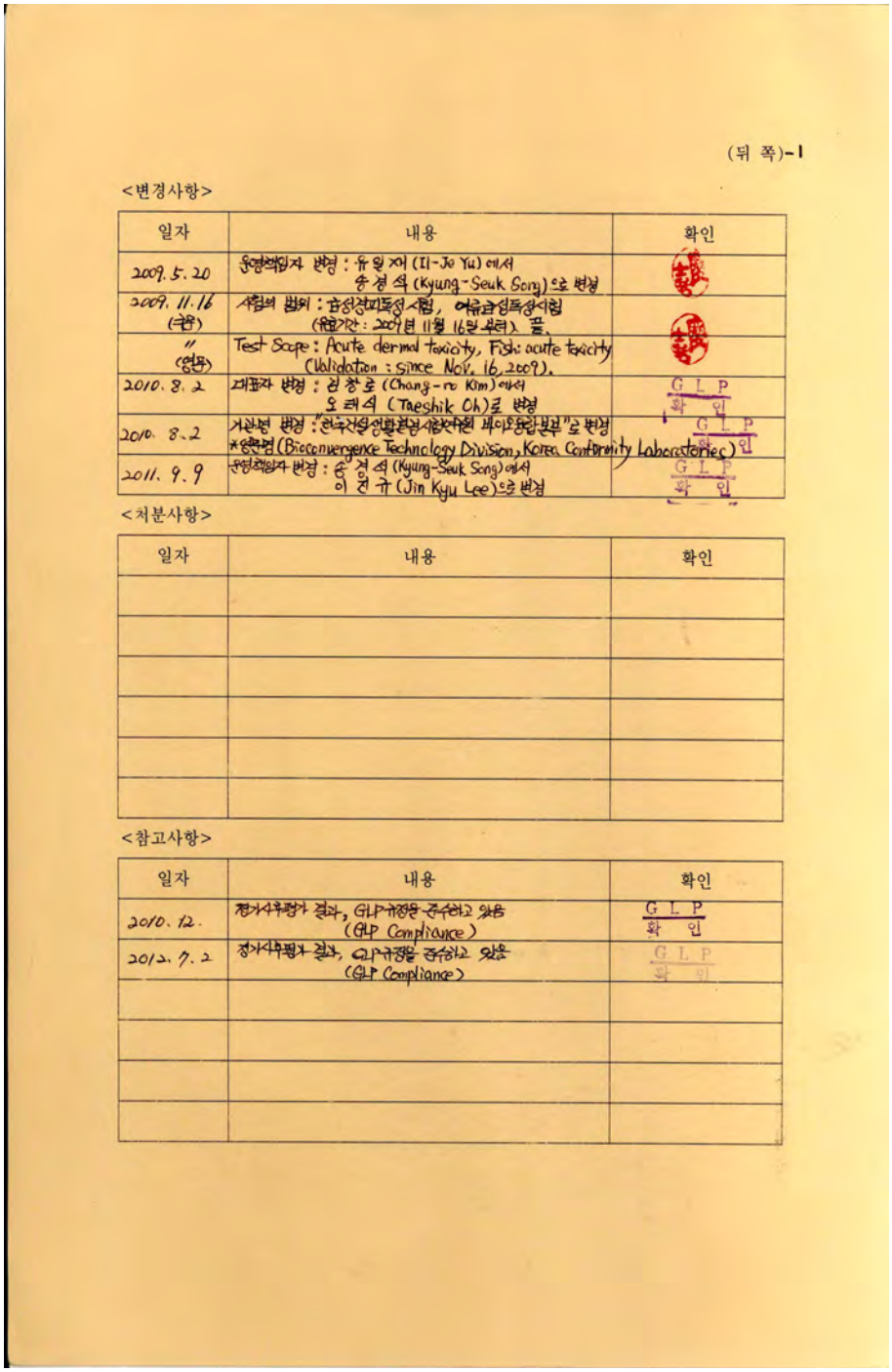
「유해화학물질관리법」 제14조, 같은 법 시행령 제12조 및 같은 법 시행규칙 제10조제2항에 따라 화학물질 유해성 시험기관(GLP시험기관)으로 지정합니다.

It is hereby certified that the test facility was inspected by the national compliance monitoring authority regarding compliance with the Principles of Good Laboratory Practice.

Issue date 2008년(year) 8월(month) 25일(date)

국립환경과학원장 인

President, National Institute of Environmental Research



(뒤 쪽)-1

<변경사항>

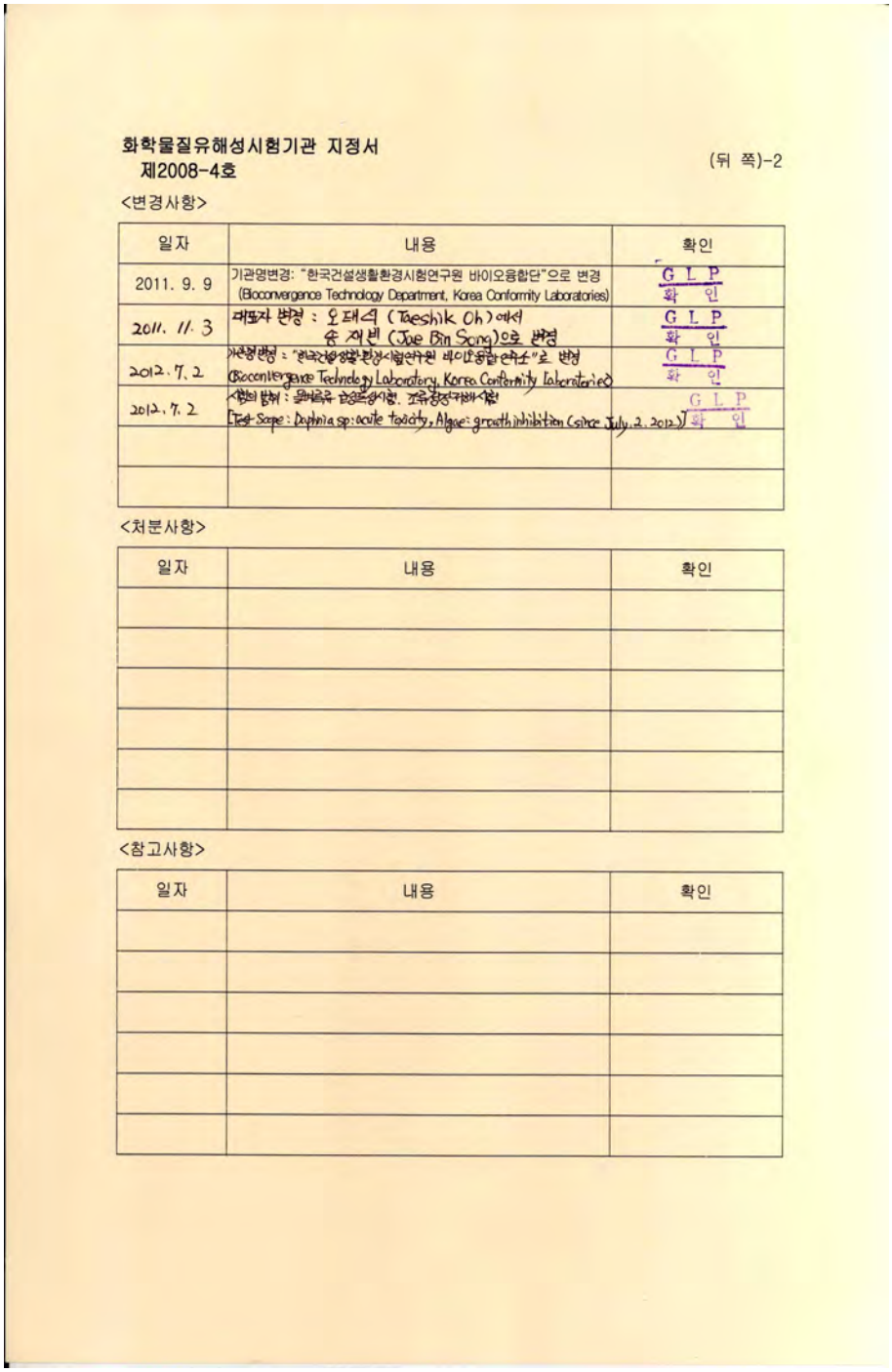
일자	내용	확인
2009. 5. 20	운영책임자 변경 : 유 일 저 (Il-Je Yu) 에서 송 경 석 (Kyung-Seuk Song) 으로 변경	
2009. 11. 16 (국문)	시험의 범위 : 급성경피독성시험, 어류급성독성시험 (유효기간 : 2009년 11월 16일 부터) 끝.	
〃 (영문)	Test Scope : Acute dermal toxicity, Fish: acute toxicity (Validation : since Nov. 16, 2009).	
2010. 8. 2	대표자 변경 : 김 창 호 (Chang-ro Kim) 에서 오 태 식 (Taeshik Oh)로 변경	G L P 확 인
2010. 8. 2	기관명 변경 : "한국지성생물융합원"에서 "바이오융합본부"로 변경 *영문명 (Bioconvergence Technology Division, Korea Conformity Laboratories)	G L P 확 인
2011. 9. 9	운영책임자 변경 : 송 경 석 (Kyung-Seuk Song) 에서 이 진 규 (Jin Kyu Lee)으로 변경	G L P 확 인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인
2010. 12.	정기사후평가 결과, GLP규정을 준수하고 있음 (GLP Compliance)	G L P 확 인
2012. 7. 2	정기사후평가 결과, GLP규정을 준수하고 있음 (GLP Compliance)	G L P 확 인



화학물질유해성시험기관 지정서
제2008-4호

(뒤 쪽)-2

<변경사항>

일자	내용	확인
2011. 9. 9	기관명변경: "한국건설생활환경시험연구원 바이오융합단"으로 변경 (Bioconvergence Technology Department, Korea Conformity Laboratories)	GLP 확인
2011. 11. 3	대표자 변경 : 오태식 (Taeshik Oh) 에서 송 진 빈 (Jae Bin Song)으로 변경	GLP 확인
2012. 7. 2	기관명변경 : "한국건설생활환경시험연구원 바이오융합연구소"로 변경 (Bioconvergence Technology Laboratory, Korea Conformity Laboratories)	GLP 확인
2012. 7. 2	시험의 범위 : 물벼룩을 이용한 급성독성시험, 조류생장저해시험 Test Scope : Daphnia sp: acute toxicity, Algae: growth inhibition (since July. 2. 2012.)	GLP 확인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인

Annex 9. Quality assurance statement-Original

신 뢰 성 보 증 확 인 서

시험번호 : GT13-00019

시 험 명 : ICR 마우스 골수세포를 이용한 MWCNT의 소핵시험

이 보고서에 기술된 시험을 독립적으로 아래와 같이 시험과정 단계별로 점검하였으며 각 점검결과를 표준작업지침서에 따라 시험책임자와 운영책임자에게 통보 및 보고하였다.

본 시험은 국립환경과학원 고시 제2012-23호(2012년 08월 22일) '화학물질 유해성시험연구기관의 지정 등에 관한 규정', 동규정 별표5 제4장 제16항 유전독성시험(골수세포 소핵시험) 및 OECD Guidelines for the Testing of Chemical No. 474에 따라 수행되었으며, 보고서의 작성 및 결과의 기술이 시험의 실시과정에서 발생한 시험기초자료를 바탕으로 정확히 반영되었음을 확인하였다.

점 검 내 용	실 시 일	시험책임자에게 통보일	운영책임자에게 보고일
시험계획서	2013. 03. 18	2013. 03. 18	2013. 03. 18
동물입수	2013. 05. 14	2013. 05. 14	2013. 05. 14
시험물질 및 대조물질	2013. 04. 01	2013. 04. 01	2013. 04. 01
시험물질조제	2013. 05. 21	2013. 05. 21	2013. 05. 21
동물사육 및 투여	2013. 05. 21	2013. 05. 21	2013. 05. 21
증상관찰 및 측정	2013. 05. 21	2013. 05. 21	2013. 05. 21
부검(골수) 및 검체제작	2013. 05. 22	2013. 05. 22	2013. 05. 22
검경	2013. 05. 22	2013. 05. 22	2013. 05. 22
시험기초자료	2013. 08. 23	2013. 08. 23	2013. 08. 23
최종보고서	2013. 08. 23	2013. 08. 23	2013. 08. 23

SOCIETY OF TOXICOLOGY Young-Seok Song 2006-02 CERTIFIED QA PROFESSIONAL IN GLP

한국건설생활환경시험연구원 바이오융합연구소
신뢰성보증책임자 송 영 석 (인)

2013년 09 월 23 일

Annex 10. Study personnel-Original

시험관계자서명

주 시험담당자	주 효 진 주시험담당자	날짜	2013 08 23
시험물질 조제	성 재 학 시험물질 조제분석 책임자	날짜	2013 [illegible]
동물관리	백 민 원 동물관리 책임자	날짜	2013.08.23
자료보관	김 효 동 자료보관 책임자	날짜	2013.8.23